ADVANCES IN IBD

Current Developments in the Treatment of Inflammatory Bowel Diseases

Section Editor: Stephen B. Hanauer, MD

Thiopurines in IBD: What Is Their Mechanism of Action?

Markus Neurath, MD
Professor of Medicine
Head of the Endoscopy Unit
Johannes Gutenberg University of Mainz
Mainz, Germany

G&H What are the different types of thiopurines?

MN There are 3 types of thiopurines that have been used for inflammatory bowel disease (IBD) therapy: azathioprine, 6-mercaptopurine, and thioguanine. The first 2 are approved for the treatment of IBD, and the third has been tested in clinical trials.

G&H What is the mechanism of action of these agents?

MN There are several factors that have been found to contribute to the development of Crohn's disease. Activation of the mucosal immune system has been found to play a key role, specifically in the chronic phase of this disease. The thiopurines are immunosuppressive drugs, deactivating key processes in T lymphocytes that lead to inflammation. The process through which azathioprine and other thiopurines treat inflammatory diseases involves a complex set of genetic activities. As our group reported in the *Journal of Clinical Investigation* in 2003, in CD4-positive T lymphocytes, azathioprine targets Rac1 activation. Rac1 is a small GTPase that is essential for activation of gut T cells.

G&H Could you describe the process by which azathioprine targets Rac1 activation?

MN Through metabolism, azathioprine is converted into 6-mercaptopurine, which is then converted into 6-thioguanine. 6-thioguanine is converted into 2 metabolites: one that is incorporated into DNA (6-thioguanine nucleotides), and one that is incorporated into small GTPases (6-thio-GTP).

Small GTPases play a role in various cell processes such as growth, differentiation, and movement. Rac1 is a member of the small GTPase protein family. We found that one of the azathioprine metabolites, 6-thioguanine triphosphate (6-thio-GTP) binds to Rac1 as a competitive antagonist of GTP. This binding suppresses the activation of Rac1, which leads to apoptosis. Essentially, through its effect on Rac1 activity, azathioprine converts a costimulatory signal into an apoptotic signal.

G&H Routine clinical use of azathioprine has shown that the benefit of this agent may take several weeks to appear. Is this timing related to the drug's mechanism of action?

MN Yes, most likely. The 6-thio-GTP has a lower affinity to Rac1 than the normal binding partner GTP. Therefore, it takes time before 6-thio-GTP–loaded Rac1 molecules accumulate in the cells and before the Rac1 inactivation takes place. This feature would explain why azathioprine has a delayed onset of clinical activity.

G&H Are these understandings about the mechanism of action behind the thiopurines influencing drug development?

MN Yes. After our group showed that azathioprine works by blocking GTPase, we turned our focus to developing more specific blocking agents to more effectively suppress the activity of GTPase.

These next-generation agents are analogs of thiopurines and are being designed to work more specifically

and more rapidly. In particular, one of the aims with this work is to create a compound that will block GTPase only, without the other metabolite that is incorporated into DNA.

G&H What are the effects of the DNA-incorporated metabolite seen with azathioprine?

MN We hypothesized that the azathioprine metabolite that is incorporated into DNA has some impact on proliferation but also carries the potential of side effects. Random incorporation into DNA will lead to random mistakes in DNA, which may cause the side effects that we see in the clinic. Eliminating this metabolite from the thiopurine analog could decrease or diminish the side effects seen with these agents.

G&H What side effects appear to be related to the DNA-incorporated metabolite?

MN Azathioprine therapy carries a risk of tumor development, particularly lymphoma. Other side effects include liver toxicity, which might be caused by DNA incorporation of metabolites.

G&H What remains to be elucidated about how the thiopurines work?

MN It is possible that there might be additional mechanisms of action. We know that azathioprine has numerous different metabolites. We cannot be sure that all of them are inactive.

G&H Does the mechanism at play ever factor into deciding which treatment is best for a given patient?

MN Not yet, but that might change in the future. We would need assays that would enable us to determine whether the Rac1 pathway is activated in a given patient. Such an assay could influence clinical outcome in the future.

G&H Could you describe how this assay might work?

MN Not all patients with Crohn's disease respond to azathioprine, which indicates that the mechanism of action described above is not being activated in some patients. Ideally, we would have an assay that could show whether the Rac1-related pathway described above is activated before a patient is treated with azathioprine.

As described above, azathioprine targets Rac1. Rac1 is the target of an exchange factor known as Vav. Azathioprine causes inactivation of Vav on the Rac1 molecule. It may be possible to develop an assay that shows whether this signaling pathway has been turned on, thereby indicating that a patient is likely to respond to azathioprine therapy.

Currently, we are working on developing such an assay, but no such system is available yet.

Suggested Reading

Atreya I, Neurath MF. Understanding the delayed onset of action of azathioprine in IBD: are we there yet? *Gut.* 2009;58:325-326.

Poppe D, Tiede I, Fritz G, et al. Azathioprine suppresses ezrin-radixin-moesin-dependent T cell-APC conjugation through inhibition of Vav guanosine exchange activity on Rac proteins. *J Immunol.* 2006;176:640-651.

Tiede I, Fritz G, Strand S, et al. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest.* 2003;111:1133-1145.

Ben-Horin S, Goldstein I, Fudim E, et al. Early preservation of effector functions followed by eventual T cell memory depletion: a model for the delayed onset of the effect of thiopurines. *Gut.* 2009;58:396-403.

Korelitz BI, Present DH. A history of immunosuppressive drugs in the treatment of inflammatory bowel disease: origins at the Mount Sinai Hospital. *Mt Sinai J Med.* 1996;63:191-201.